TED ANKARA COLLEGE FOUNDATION HIGH SCHOOL

Comparison of the Probiotic Effects of *Lactobacillus acidophilus* and *Enterecoccus faecalis* as indicated by autoaggregation and coaggregation percentages

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Abstract:

The aim of this extended essay was to compare the probiotic penetrances of *Lactobacillus aciophilus* and *Enterecoccus faecalis* which is indicated by percentages of autoaggregation and coaggregation.

The research question was "How do the probiotic effects of *Lactobacillus acdiophilus* and *Enterecoccus faecalis* differ from each other as indicated by autoaggregation and coaggregation percentages?".

It was hypothesized that "*L.acidophilus* will have higher percentages of autoaggregation and coaggregation, which shows that *L.acidophilus* has higher probiotic effect compared to *E.faecalis*." To test this hypothesis, a common procedure, Vandervoorde et. al. (1992) was used with some modifications to conduct the aggregation and coaggregation assays. For the coaggregation assay *E.coli* is needed besides *L.acidophilus* and *E.faecalis* as *E.coli* is prime for the human gut flora. The autoaggregation and coaggregation results were calculated by a formula using optical densities measured by spectrophotometer.

The average percentage of autoaggregation for *L.acidophilus* is 82 % and average percentage of coaggregation for *E.faecalis* is 20.2 %. When average percentage of coaggregation is taken into consideration it is seen that coaggregation percentage of *L.acidophilus* is higher than *E.faecalis*. Average percentage of coaggregation for L.acidophilus is 65 % and for E.faecalis it is 7.5 %. The statistical analysis was done by using one-tailed t-test. The p values were found to be 2,55119E-10 for autoaggregation and 2,51E-11 for coaggregation. Since both p values were below 0.05, the hyptohesis was supported. *L.acidophilus* has higher percentages of autoaggregation and coaggregation and this results indicate that *L.acidophilus* has more probiotic penetrance than *E.faecalis* since percentages of autoaggregation and coaggregation are criteria for selecting probiotic bacteria.

Word Count: 259

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Introduction:

In my daily life I have always loved eating yogurt like many other people in the world. Yoghurt is a dairy product which is produced by the fermantation of milk. The lactic acid produced as a result of this process acts on milk protein and gives yoghurt its characteristic tang. All around the world yoghurt is a highly favored appetizer. For example in Turkish culture, cacık is a common appetizer which we consume a lot. Untill 1900s yoghurt was a staple in the diets of people such as Russian Emperors. Yoghurt is not only delicious food, but it is also nutritionally valuable since it is rich in protein, calcium, riboflavin, vitamin B6 and vitamin B12.[1] It is a very important part of children's diet. In an attempt to find a solution to digestive problems arising from our altering nutritional habits, yoghurt manufacturers have developped varieties of yoghurt prepared by adding probiotic bacteria to traditional yoghurt. This change made me interrogate what probiotic bacteria are, why we need such special kinds of bacteria and whether the probiotic effect of bacteria is same for all or not. Increasing usage of probiotic bacteria made them appear in the advertisments more frequently, redounding my curioisty even more.

A probiotic is generally defined as "a live micro-organism which, when administered in adequate amounts, confers a health benefit on the host"[2] Probiotic bacteria have possitive effects on human metabolism and health. We need these bacteria since in our daily life, we do not have enough time to settle an organic and healthy meal time. We just eat what we get without looking if it is good for our health or not. That kind of unhelathy nutrition, mostly eating fastfood or else, affects human digestive sytem and of course overall health badly. At this point, the reason why we need probiotic bacteria comes to the scene. Probiotic bacteria play a crucial role in especially at the gut flora in order to balance bacterial homeostasis and protect human metabolism from pathogens and diseases such as diarrhoea, lactose intolerance, colon cancer, cholesterol and inflammation.[3] Use of probiotic bacteria also have a positive economic impact in terms of protecting human health. Probiotic bacteria help digestive system as they maintain bacterail homeostasis at the gut flora and they have positive effects on overall health. So, taking probiotic food as nutrients decreases the amount of money that is spent to medications in order to maintain ones good health condition. Moreover, according to a Turkish yogurt manufacturer, probiotic bacteria have some psychological effects as they ease the digestive tract. A survey that has been made by this company reveal that 12 % of

[1] http://en.wikipedia.org/wiki/Yogurt
[2] FAO and WHO 2002
[3] http://en.wikipedia.org/wiki/Probiotic

people who suffer from digestive problems can not focus on their daily work and 18% of people can not concentrate on their business life.[4]

There are numerous types of bacteria which can be considered as probiotic and can be used in probiotic nutrients. Review of literature concerning food industry showed that, especially for yogurts, two of the most common ones are types of *Lactobacillus* and *Enterecoccus*. Different brands use different bacteria in their products but why does the type of bacteria change according to product? Criteria for selection of probiotic bacteria include lack of pathogenicity, tolerance to gastro intestinal conditions, ability to adhere the gastro intestinal mucosa, competitive exclusion of pathogens, and percanteges of autoaggregation and coaggregation. Autoaggregation is related with the cell-cell adhesion between bacteria of the same strain. Coaggregation is cell-cell adhearence between different bacteria posterities.[5] Coaggregation of a bacterium takes place by clustering of the probiotic with *Escherichia coli*, a bacterium which is prime to the gut flora.[6] In order to protect digestive tract from pathogens, a probiotic bacterium needs to have high percentages of autoaggregation and coaggregation, indicating that it covers up more space in the gut flora. Therefore, a probiotic is a kind of bacteria which is considered as live microorganism that confers a health benefit to host.

Related research showed that two of the most commonly used probiotic bacteria in food industry are *Lactobacillus acidophilus* and *Enterecoccus faecalis*. Therefore, in this practical I decided to compare the probiotic effects of these two bacteria. *Lactobacillus acidophilus* is the latin name for milk loving bacteria which gives us clue about why this bacteria is chosen the most. Moreover, *L.acidophilus* works at mediums which has pH lower than 5.0. This property of *L.acidophilus* indicates that it is suitable for the human digestive system's acidic property. *L.acidophilus* also, help digestive system by breaking down nutirents; it protects the intestines and even can prevent diarrhea.[7]

On the other hand, *Enterecoccus faecalis* is a Gram-positive bacteria which may cause life threatening infections for humans. *E.faecalis*, inhibits human gastrointestinal tract but it is among the constituents of probiotic bacteria.[8] Some strains of *E.faecalis* can be found in water and soil but there are some other types that are found at vaginal and intestinal regions of human. *E.faecalis*

[4] http://www.activiaturkiye.com/sindirim_sistemimiz/saglikli_sindirimsistemine_ihtiyac.aspx

[5] http://books.google.com.tr/books?id=ohZtUQZ4uHwC&pg=PA386&lpg=PA386&dq=what+%C5%9Fs+autoaggregation+and+coaggregation&source=bl&ots=aFTCa3lpNZ&sig=kbbzzkEjqlJnWoc8cIUzuP72xAc&hl=en&sa=X&ei=dh9ET-HnK8Sl0QWuw5iPDw&redir_esc=y#v=onepage&q=what%20%C5%9Fs%20autoaggregation%20and%20coaggregation&f=false

[6] Adhesion and aggregation properties of probiotic and pathogen strains Maria Carmen Collado · Jussi Meriluoto · Seppo Salminen Springer-Verlag 2007

[7] http://www.umm.edu/altmed/articles/lactobacillus-acidophilus-000310.htm

[8] http://en.wikipedia.org/wiki/Enterococcus_faecalis

prevents colonization of pathogenic bacteria in the body. It may also prime the immune system by inducing the production of low levels of antibodies against its own components which, in turn, makes the immune system more efficient. Moreover, characteristics of *E.faecalis* can be used for the production of probiotics which are considered as dietary suplements and nutrients that help treat conditions like irritable bowel syndrome and vaginal infections. *E.faecalis* strains play a crucial role in the dairy industry and present in variety of cheeses, whey and milk. To continue with*, E.faecalis* secretes an enzyme known as bacteriocin that can inhibit the growth of some pathogenic bacteria such as vibrio chlorea.[9]

According to the information given above, my main objective is to show whether *L.acidophilus* or *E.faecalis* has a higher probiotic effect as indicated by higher autoaggregation and coaggregation percentages. For this purpose, *L.acidophius'* and *E.faecelis'* autoaggregation and coaggregation percentages will be compared and the result will light us through the most probiotic bacteria among these two. For the coaggregation assay, cell-cell adhesion of *L.acidophilus* with *E.coli* and *E.faecalis* with *E.coli* will be investigated. Therefore, the research question that will be studied in this essay is "How do the probiotic effects of *L.acdiophilus* and *E.faecalis* differ from each other as indicated by autoaggregation and coaggregation percentages?"

Hypothesis:

Probiotic bacteria are human friendly type among the bacteria. There are numerous studies done about probiotic bacteria and their affects on human health. For example Yavuz Beyatlı evaluates the effects of probiotic bacteria at the metabolic activities at his study *"Assessment of Potential Probiotic Properties of Lactobacillus spp. , Lactococcus spp. , Pediococcus spp. Strains Isolated From Kefir"* This study demonstrates high probiotic penetrance for almost all *Lactobacillus* types.[10] Nowadays probiotic bacteria, including *L.acidophilus* and *E.faecalis* that are the subjects of this study, are one of the main interests of biologists working in the field of food industry and fabricates probiotic foods especially probioitc yogurts. In literature, there is enough evidence that probiotic bacteria are human friendly and beneficial to humans in terms of medical costs and stressful daily business lives' bad affects on human digestive system.

[9] http://www.livestrong.com/article/244675-beneficial-uses-of-enterococcus-faecalis

[10] *Assessment of Potential Probiotic Properties of Lactobacillus spp. , Lactococcus spp. , Pediococcus spp. Strains Isolated From Kefir*

According to this phenomenon, this practical has been planned to compare probiotic penetrances of two commonly used bacteria by comparing their percentages of autoaggregation and coaggregation.

In this study it is expected that, autoaggregation and coaggregation percentages of *L.acidophilus* and *E.faecalis* indicates their probiotic penetrance when compared at the same medium, Man Ragosa medium, at constant pressure, temperature and pH. The hypothesis of this study is that "*L.acidophilus* will have higher percentages of autoaggregation and coaggregation, which shows that *L.acidophilus* has higher probiotic effect compared to *E.faecalis*."

Method Development And Planning:

My research question is "How do the probiotic effects of *L.acdiophilus* and *E.faecalis* differ from each other as indicated by autoaggregation and coaggregation percentages?" When I first began studying about probiotic nutrients, I intended to investigate the effects of probiotic yogurts on digestive activity. However, the high number of different variables and complications I faced about finding the right instrument to use made me change my mind. Later on, I decided to investigate the probiotic bacteria that are present in probiotic nutrients. Isolating my own bacterial strains was a better but harder way to find the relation between probiotic penetrance and percentages of autoaggregation and coaggregation.

There are several ways of isolating bacteria strains and finding their percentage of autoaggregation and coaggregation. The procedure I chose for both aggregation assays was the one which was described by Vandeervorde et al*. (1992)* [11] with some modifications. This procedure allows obtaining quick results and it is a common procedure applied by many institutions including Gazi University where I had technical and labaratory support from Assistant Proffessor Dr. Zehra Nur Yuksekdağ. Using a common procedure is efficient in terms of time since the nutritional media used for growing bacteria are readily available. According to this procedure, bacteria are allowed to aggregate in petri plates, and percentages of autoaggregation and coaggregation are calculated using first optical density and second optical density after 4 hours. Optical densities are measured using a spectrophotometer.[12] One obstacle is that bacteria may not aggregate properly. To overcome this obstacle inversion of light microscope will be used to observe suspensions for aggregation.

Being researched the probiotic bacterial ingredients of different kinds of nutrient, I decided comparing *Lactobacillus acidophilus* and *Enterecoccus faecelis* since they are two of the most frequently used bacteria for probiotic nutrients. These bacteria may be isolated from hand made yogurt or else. However there is a risk of implicate *L.acidophilus* and *E.faeecalis* with any other bacteria. Growing bacteria from commercially available samples will eliminate this risk.

In order to grow bacterial cultures Man, Ragosa Sharpe (MRS) medium at 37^{o}C is choosen rather than agar. Although agar is a mainstream medium used very often, and preparation of MRS medium is very time consuming due its critical propotions of chemicals, MRS medium is still more preferable

[11] The Lactic Acid Bacteria: The Genera Of Lactic Acid Bacteria; Brian J. B. Wood, W. H. Holzapfel 1995

[12] SOME FACTORS AFFECTING THE AUTOAGGREGATION ABILITY OF VAGINAL LACTOBACILLI ISOLATED FROM TURKISH WOMEN HAVVA EKMEKÇİ1, BELMA ASLIM1, and DERYA ÖNAL DARILMAZ2 1Department of Biology, Faculty of Science and Arts, Gazi University, Teknikokullar, 06500 Ankara, Turkey, 2Department of Biology, Faculty of Science and Arts, Aksaray University, 6800 Aksaray, Turkey.

since it permits faster bacterial growth. Moreover, the constituents of MRS medium, which are indicated in appendix 1, are more suitable than agar for autoaggregation assays and gives more precise results. 37^{o}C is the optimum temperature especially for *L.acidophilus*. To stabilize temperature untill harvesting of bacteria, incubator will be used. Three petri plates will be prepared for each bacterium.

As indicated in the introduction, *E.coli* is selected for coaggregation assays, as *E.coli* is prime bacteria for human gut flora. Altough MRS medium is selected for the growth of probiotic bacteria, agar broth medium will be used for growing *E. coli* since it is easier to prepare and medium selection is not so critical for this bacteria.

Bacteria strains are harvested by centrifugation at 10.000 x gravitational acceleration for 15 minitues and washed twice with distilled water as a common procedure. Using centrifuge as a harvest method was the only option while growing bacteria from commercially available samples.

Since this study involves use of bacteria, working in sterile conditions is very important. To prevent contamination, all stages involving transfer of bacteria should be conducted near a bunsen burner. A disadvantage of using bunsen burner is that, high temperature may cause denaturation of bacteria. To prevent this, bacteria will be subjected to heat and lipase treatments to render them resistant to temperature and pH changes.[13] Heat treatment involves exposure to high temperatures for 20 minutes at 85^{0}C and 30 minutes at 70^{0}C. Lipase treatment is performed in PBS, pH 7.5.

The autoaggregation assay:

Autoaggregation assays will be performed according to the method of Vandervoorde et al. (1992). After harvesting, *L.acidophilus* and *E.faecalis* will be washed with PBS solution, the contents of which are explained in appendix 2, and their optical densities are obtained by spectrophotometer at 600 nm.

The coaggregation assay:

Coaggregation assays will be performed according to the method of Vandervoorde et al. (1992). Coaggregation percentage of a bacteria is found with the formula written in method section. The coaggregation of a probiotic bacteria is related with the rate of clustering with *E.coli* as gut flora has

[13] http://www.enotes.com/bacterial-resistance-response-antibacterial-agents-reference/bacterial-resistance-response-antibacterial-agents

been taken basis for the investigation of coaggregation. At this experiment, variables that are needed to calculate the percentage of coaggregation is the optical densities of probiotic bacteria. Optical densities of probiotic bacteria will be measured with spectrophotometer at 600 nm.

The bacteria used for assays:

In both autoaggregation and coaggregation assays *L.acidophilus* strain Z12 and *E.faecalis* strain NCDO 581 are used. High percentage of coaggregation means better probiotic penetrance and less pathogens at the gut flora. The difference between the percentages of autoaggregation and coaggregation of *L.acidophilus* and *E.faecalis* makes the comparison of their probiotic penetrance easier and these two bacteria strains are accepted to be convenient for probiotic penetrance.

In this practical my constant variables were temperature, pH and pressure. Conducting the experiment in labaratory conditions ensures that these variables are kept constant. To start with, general heating system of the labaratory keeps room temperature constant at 24.0^0C, which is good for human physiology and not too high to cause denaturation of bacterial enzymes. Secondly, the pressure measured was constant as the location of labaratory has not been changed. Finally, the PBS solution that will be prepared is going be adjusted to a pH value of 6 which will be kept constant using an attentively calibrated pH probe.

L.acidophilus and *E.faecalis* strains will grow for 18-20 hours which is the time needed for bacterias exponential growth phase at 37^oC. Bacterial strains are stored at – 80^0C untill aggregation and coaggregation assays. The reason for storing bacterial strains at really low temperatures like – 80^0C is that frozen stocks of bacterial strains can survive for long term experiments.

At this practical I decided to make five trials for each aggregation type(autoaggregation and coaggregation). Five trials will be enough to avoid any random errors and will be a managable size taking time constraints into consideration.

Materials:

- Centrifuge
- Light Microscope
- Phosphate Buffer (1 liter pH 6.0)
- PBS (1 M NaOH, 1M HCl)
- Spectrophotometer
- Vortex
- Micropipette
- Bunsen burner
- pH probe
- Pasteur oven
- Incubator
- Lam
- Rack
- Pure strain (L.acidophilus, E.faecalis)
- Autoclave
- Gram Strain
- Test tube x25
- General labaratory glassware ie, graduated cylinder
- Gloves
- Mask
- Water purification system
- Agar nutrient broth

Method:

Gloves and mask must be worn during the procedure of this practical to prevent contamination risks and health issues.

Procedure:

A. *L.acidophilus* and *E.faecalis* strains were grown and isolated according to the procedure mentioned at the early parts of this essay at the Labaratory of Biology Faculty, Gazi University. The isolated strains kept – 80^0C, to maket hem survive throughout the experiment. Heat and lipase treatment were performed before autoaggregation and coaggregation assays in order to compose resistance.

B. Autoaggregation and coaggregation assays were performed.

1. Prepare Man, Rogosa and Sharpe medium (MRS).
2. L.acidophilus and E.faecalis bacteria strains were grown at MRS medium for 18-20 hours at 37^oC.
3. After growth of bacteria, the isolates were stored at $–80^o$C in MRS.
4. Both bacteria strains *L.acidophilus* and *E.faecalis* were subcultured twice before use.
5. Perform heat and lipaase treatment to bacteria in order to compose resistance to heat and pH change.
6. Activated cultures were harvested by centrifugation for 15 min at 10.000 x g
7. Activated cultures washed twice with PBS(pH 6.0) to give optical density.
8. Optical density was measured by spectrophotometer (Hitachi U1800) at 600 nm.
9. For autoaggregation sample consisted of 2 ml of each strain, OD was measured at 600 nm.
10. The percentages of autoaggregation and coaggregation was expressed as follows:
    - ✓ Autoaggregation % = [$(OD_1 – OD_2) / (OD_1)$ x 100]

    OD_1: First optical density, OD_2: Second optical density after 4 h.

    All of the suspensions were obsserved by inversion light microscopy.
    - ✓ Coaggregation % = [$(OD_1 + OD_2) – 2(OD_3) / (OD_1+OD_2)$ x 100]

    OD_1: First optical density strain 1 *(Lactobacillus acidophilus),* OD_2: Second optical density strain 2 *(Enterecoccus faecalis),* OD_3: Optical density of strain 1 and 2.

11. Cultures that are harvested by centrifugation were washed by distilled water.

12. After several different treatments like heat treatment, lipase treatment(pH 7.5) etc.
13. Bacterial cells were examined for autoaggregation at different pH values ranging from 3 to 9.
14. Each of these steps were followed 5 times for each strain as five trials were made.

Results:

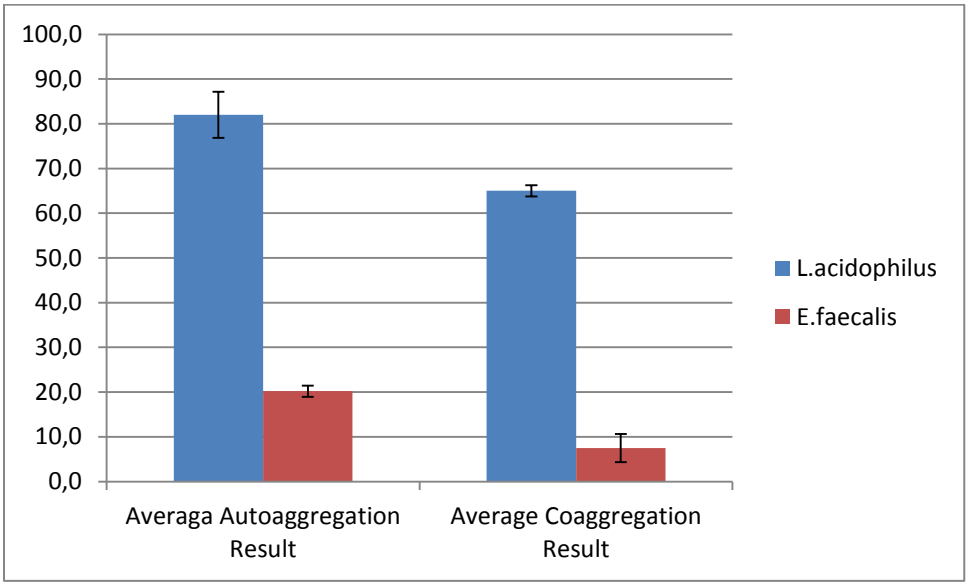
Results obtained from the autoaggregation and coaggregation assays are expressed in the following tables and graphs.

Type of bacterial strain	% Autoaggregation Results ±0.2	% Coaggregation Results ±0.2	Environmental Pressure (Pa)± 0.5	Temperature (°C) ±0.5	pH of medium ±0.5
Lactobacillus acidophilus	82,0	62,0	1014,0	24,0	6,0
	79,0	64,0	1014,0	24,0	6,0
	85,0	65,0	1014,0	24,0	6,0
	77,0	65,0	1014,0	24,0	6,0
	87,0	69,0	1014,0	24,0	6,0
Enterecoccus faecalis	17,0	9,0	1014,0	24,0	6,0
	15,0	8,0	1014,0	24,0	6,0
	16,0	7,0	1014,0	24,0	6,0
	15,0	7,0	1014,0	24,0	6,0
	17,0	9,0	1014,0	24,0	6,0

Table 1: Type of bacterial strain, autoaggregation results, coaggregation results, environmental pressure, temperature, pH of medium.

Type of bacterial strain	% Average Autoaggregation Result ±0.2	% Average Coaggregation Result ±0.2
L.acidophilus	82,0	65,0
E.faecalis	20,2	7,5

Table 2: Type of bacterial strain, average autoaggregation result, average coaggregation result, environmental pressure, temperature, pH of medium.



Graph 1: Average autoaggregation and coaggregation percentages obtained from the formula that been expressed.

L.acidophilus		*E.faecalis*	
Mean	82	Mean	16
Standard Error	1,843908891	Standard Error	0,447213595
Median	82	Median	16
Standard Deviation	4,123105626	Standard Deviation	1
Sample Variance	17	Sample Variance	1
Minimum	77	Minimum	15
Maximum	87	Maximum	17
Sum	410	Sum	80
Count	5	Count	5
Confidence Level(95,0%)	5,119511816	Confidence Level(95,0%)	1,241663998

Table 3: Statistical analysis of % autoaggregation resultsof L.acidophilus and E.faecalis.

Mean	65	Mean	8
Standard Error	1,140175425	Standard Error	0,447213595
Median	65	Median	8
Standard Deviation	2,549509757	Standard Deviation	1
Sample Variance	6,5	Sample Variance	1
Minimum	62	Minimum	7
Maximum	69	Maximum	9
Sum	325	Sum	40
Count	5	Count	5
Confidence Level(95,0%)	3,165634478	Confidence Level(95,0%)	1,241663998

Table 4: Statistical analysis of % coaggregation results of L.acidophilus and E.faecalis

Data Analysis:

Statistical analysis is performed by Excel 2010 computer software.

	% Autoaggregation Results of L.acidophilus	*% Autoaggregation Results of E.faecalis*
Mean	82	16
Variance	17	1
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	34,78505426	
P(T<=t) one-tail	2,55119E-10	
t critical one-tail	1,859548038	

Table 5: t-test result for % Autoaggregation results of L.acidophilus and E.faecalis

Null hypothesis (H0): The difference between the average autoaggregation percentages of L.acidophilus and E.faecalis is not statistically significant, since it is not large enough to be explained by chance only.

Alternative hypothesis (HA): The difference between the average autoaggregation percentages of L.acidophilus and E.faecalis is statistically significant, since it is too large to be explained by chance only.

H_0 is rejected as pvalue=2,55119E-10 < 0.05

	%Coaggregation Result of L.acidophilus	*%Coaggregation Result of E.faecalis*
Mean	65	8
Variance	6,5	1
Observations	5	5
Hypothesized Mean Difference	0	
df	8	
t Stat	46,54031	
P(T<=t) one-tail	2,51E-11	
t critical one-tail	1,859548	

Table 6: t-test result for % Coaggregation result of L.acidophilus and E.faecalis

Null hypothesis (H0): The difference between the average coaggregation percentages of L.acidophilus and E.faecalis is not statistically significant, since it is not large enough to be explained by chance only.

Alternative hypothesis (HA): The difference between the average coaggregation percentages of L.acidophilus and E.faecalis is statistically significant, since it is too large to be explained by chance only.

H_O is rejected as p=2,51E-11< 0.05

P<0.05 it indicates a significant difference between the probiotic penetrance of *L.acidophilus* and *E.faecalis*.

Evaluation:

The aim of this study was to compare the probiotic penetrance of two bacteria, *L.acidophilus* and *E.faecalis.* The research question "How do the probiotic effects of *L.acdiophilus* and *E.faecalis* differ from each other as indicated by autoaggregation and coaggregation percentages?". It was hypothesized that "*L.acidophilus* will have higher percentages of autoaggregation and coaggregation, which shows that *L.acidophilus* has higher probiotic effect compared to *E.faecalis*."

To test this hypothesis, optical densities were calculated by using spectrophotometer; percentages of autoaggregation and coaggregation of L.acidophilus and E.faecalis were calculated via the formulae expressed in the procedure part. Average autoaggregation percentages were found to be 82 % for *L.acidophilus,* and 20.2 % for *E.faecalis*. Average percentages of coaggregation on the other hand, were 65 % for *L.acidophilus* and 7.5 % for *E.faecalis.* These differences in average percentages of autoaggregation and coaggregation indicate a difference in their probiotic penetrance. Because, a bacteria needs to have high percentages of autoaggregation and coaggregation in order to cover the gut flora and be useful for human health. The differences of *L.acidophilus* and *E.faecalis* are clearly stated in *Graph 1.* To determine the statistical significance of these values, one tailed t-test was conducted and the results are indicated in *tables 5 and 6.*

The null hypothesis was that, the probiotic effects of *L.acidophilus* and *E.faecalis* differ from each other as indicated by autoaggregation and coaggregation percentages. From the statistical analysis, t-tests, performed with Excel 2010 the p values obtained were 2,55119E-10 for autoaggregation and 2,51E-11 for coaggregation, both of which are smaller than 0.05 indicating statistical significance of the results. Since autoaggregation and coaggregation results indicate a bacterium's probiotic penetrance, *L.acidophilus* is shown to be more probiotic among the two bacteria chosen.

While doing autoaggregation and coaggregation assays there weren't any unexpected occurances that may have affected the result of autoaggregation and coaggregation assays. However, there are some error sources and limitations that should be taken into consideration.

The use of autoclave was very critical, since the bacterial suspensions have to be strerilized for obtaining accurate results. Use of Bunsen Burner is also important for the same reason. You needed to be real close to Bunsen Burner in order to minimize the risk of external contamination. Any contaminant may result in deviations in autoaggregation and coaggregation assays.

One of the most important variables in the experiment was pH, which was kept constant at 6 using an attentively calibrated pH probe. However, pH still showed some deviation, approximately ± 0.2,

during the addition of chemicals used in PBS solution. Although it has been stiffed again, this minor change may have caused changes in bacterial activity. To overcome this problem, a buffer may be used to stabilize pH.

Autoaggregation and coaggregation percentages which are used as indications of probiotic penetrance are measured in vitro in this study. However, the effects of probiotic bacteria is well known, process of coaggregation assay at the human gut flora could not be demonstrated because it will not be ethic for a high school student to use human at this practical. As this study was not demonstrated in human gut flora, the result may have differ with the condition that this study has been done via using human gut flora.

In this assay only the probiotic penetrance according to the results of percentages of autoaggregation and coaggregation examined not all the elements that a probiotic bacteria should have. Checking the other elements of probiotic bacteria, in order to compare the probiotic penetrance, was exceeding my knowledge and my abilities.

Altough, the result of my experiment does not contradict with other experiments done on probiotic bacteria, my experiment could not be generalized because two specific bacteria were chosen, *L.acidophilus* and *E.faecalis*, which I decided with the guidance of my researches about ingredients of probiotic nutrients.

For further repetitions, any other different type of bacteria should be use in order to state the hypothesis that percentages of autoaggregation and coaggregation results indicates a bacterium’s probiotic penetrance.

Conclusion:

My research question “How do the probiotic effects of *L.acdiophilus* and *E.faecalis* differ from each other as indicated by autoaggregation and coaggregation percentages?” is found its answer in the light of these results my study. Percentages of autoaggregation and coaggregation results of *L.acidophilus* and *E.faecalis* indicates their probiotic penetrance which is ecpecting and approves my hypothesis. Percentage of aggregation and coaggregation results of *L.acidophilus* shows that *L.acidophilus* is a better probiotic bacteria when compared with *E.faecalis*.

The reason why I chose this subject as my extended essay subject is that in today’s World lots and lots of people started talking about the probiotic nutirients especially yogurts which are said to be health and digestive friendly. I chose to compare probiotic penetrance due to two different bacteria’s percentages of autoaggregation and coaggregation results because they need to aggregate and cover the human gut flora in order to be effective. The extend of this study was over my abilities so I decided to limit this study with two different types of bacteria an one element of probiotic criteria. Altough, there are lots of studies comparing probiotic penetrance of bacteria, my essay differs with the limitations that have been put.

As the features of this practical is limited, this sould not be interpreted as only the percentages autoaggregation and coaggregation results indicates its probiotic character. Altough, my hypothesis is supported by scientific datas, other elements of probiotic bacteria should be tested in order to make a better comparison.

Probiotic nutrition is a trend becoming more and more popular each day as the science proves the benefits of probiotic nutrition. In this case, probiotic yogurts are getting important for humans as they are maintain bacterial balance of human digestive track and help to maintain better health. Due to my assays based on aggregation percentages using *L.acidophilus* in yogurts will result better in terms of *L.acidophilus’* better probiotic penetrance. The question “ Is there any other bacteria type with different character than *L.acidophilus* that will result with better consequence? “ is still needs to be investigated.

Appendix 1:

Composition of Man. Rogosa Sharpe Medium:[14]

Ingredients	Grams/Litre
1.Peptone	10.0
2.Meat extract	8.0
3.Yeast extract	4.0
4.D(+)-Glucose	20.0
5.Dipotassium hydrogen phosphate	2.0
6.Sodium acetate trihydrate	5.0
7.Triammonium citrate	2.0
8.Magnesium sulfate heptahydrate	0.2
9.Manganous sulfate tetrahydrate	0.05

[14] http://www.sigmaaldrich.com/etc/medialib/docs/Fluka/Datasheet/69966dat.Par.0001.File.tmp/69966dat.pdf

Appendix 2:

Composition of PBS Solution:

1. Distilled water 800 ml.
2. NaCl 8 gram.
3. KCl 0.2 gram
4. Na_2HPO_4 1.44 gram.
5. KH_2PO_4 0.24 gram.
6. Adjust pH to 7.5 with sufficient amount of HCl.

Bibliography:

Adhesion and aggregation properties of probiotic and pathogen strains Maria Carmen Collado · Jussi Meriluoto · Seppo Salminen Springer-Verlag 2007

Assessment of Potential Probiotic Properties of Lactobacillus spp. , Lactococcus spp. , Pediococcus spp. Strains Isolated From Kefir

FAO and WHO 2002

The Lactic Acid Bacteria: The Genera Of Lactic Acid Bacteria; Brian J. B. Wood, W. H. Holzapfel 1995

SOME FACTORS AFFECTING THE AUTOAGGREGATION ABILITY OF VAGINAL LACTOBACILLI ISOLATED FROM TURKISH WOMEN HAVVA EKMEKÇİ1, BELMA ASLIM1, and DERYA ÖNAL DARILMAZ2 1Department of Biology, Faculty of Science and Arts, Gazi University, Teknikokullar, 06500 Ankara, Turkey, 2Department of Biology, Faculty of Science and Arts, Aksaray University, 6800 Aksaray, Turkey.

http://en.wikipedia.org/wiki/Yogurt

http://en.wikipedia.org/wiki/Probiotic

http://www.activiaturkiye.com/sindirim_sistemimiz/saglikli_sindirimsistemine_ihtiyac.aspx

http://books.google.com.tr/books?id=ohZtUQZ4uHwC&pg=PA386&lpg=PA386&dq=what+%C5%9Fs+autoaggregation+and+coaggregation&source=bl&ots=aFTCa3lpNZ&sig=kbbzzkEjqlJnWoc8cIUzuP72xAc&hl=en&sa=X&ei=dh9ET-HnK8Sl0QWuw5iPDw&redir_esc=y#v=onepage&q=what%20%C5%9Fs%20autoaggregation%20and%20coaggregation&f=false

http://www.umm.edu/altmed/articles/lactobacillus-acidophilus-000310.htm

http://en.wikipedia.org/wiki/Enterococcus_faecalis

http://www.livestrong.com/article/244675-beneficial-uses-of-enterococcus-faecalis

http://www.enotes.com/bacterial-resistance-response-antibacterial-agents-reference/bacterial-resistance-response-antibacterial-agents

http://www.sigmaaldrich.com/etc/medialib/docs/Fluka/Datasheet/69966dat.Par.0001.File.tmp/69966dat.pdf